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Effects of ELF (Extremely Low Frequency) (1-120 Hz) and Modulated (50 Hz) RF (Radio Frequency) Fields on the Efflux of Calcium Ions from Brain Tissue In vitro

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C. Blackman, S. Benane, D. House, and W. Joines

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16. ABSTRACT

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Effects of ELF (1–120 Hz) and Modulated (50 Hz) RF Fields on the Efflux of Calcium Ions From Brain Tissue In Vitro

C.F. Blackman, S.G. Benane, D.E. House, and W.T. Joines

Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina

We have previously shown that 16-Hz, sinusoidal electromagnetic fields can cause enhanced efflux of calcium ions from chick brain tissue, in vitro, in two intensity regions centered on 6 and 40 $V_{p\text{-}p}$/m. Alternatively, 1-Hz and 30-Hz fields at 40 $V_{p\text{-}p}$/m did not cause enhanced efflux. We now demonstrate that although there is no enhanced efflux associated with a 42-Hz field at 30, 40, 50, or 60 $V_{p\text{-}p}$/m, a 45-Hz field causes enhanced efflux in an intensity range around 40 $V_{p\text{-}p}$/m that is essentially identical to the response observed for 16-Hz fields. Fields at 50 Hz induce enhanced efflux in a narrower intensity region between 45 and 50 $V_{p\text{-}p}$/m, while radiofrequency carrier waves, amplitude modulated at 50 Hz, also display enhanced efflux over a narrow power density range. Electromagnetic fields at 60 Hz cause enhanced efflux only at 35 and 40 $V_{p\text{-}p}$/m, intensities slightly lower than those that are effective at 50 Hz. Finally, exposures over a series of frequencies at 42.5 $V_{p\text{-}p}$/m reveal two frequency regions that elicit enhanced efflux—one centered on 15 Hz, the other extending from 45 to 105 Hz.

Key words: ELF fields, radiofrequency radiation, amplitude modulation, 60 Hz, calcium-ion efflux, brain tissue, frequency windows, intensity window

INTRODUCTION

Internally generated electric fields and currents, in addition to their recognized fundamental role in neural activity, are also involved in some way as agents that guide development, differentiation, and repair [Liboff and Rinaldi, 1974]. Because the influence of electric fields on these processes has not been fully identified and characterized, there remains the underlying question of whether externally imposed, low-intensity electric and magnetic fields can intervene and alter these normal biological processes. There are conflicting reports concerning changes induced by electromagnetic fields at many frequencies over the spectrum from zero to 300 GHz. For a review of this subject, see Gandhi [1980]. The biological response induced by exposure to electromagnetic radiation at radio and microwave frequencies and at high intensities is generally considered to involve temperature elevation caused by the

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Address reprint requests to C.F. Blackman, Health Effects Research Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711.

deposition and subsequent thermalization of electromagnetic energy in the sample. There are other changes, occurring at lower intensities and in narrower frequency ranges, that defy this explanation [for example, Taylor, 1981]. One of the more robust biological responses generally recognized to be divorced from changes caused by temperature elevation is the phenomenon of field-induced changes in the association of calcium ions with brain tissue in vitro. This report will focus on identifying some of the electromagnetic-field conditions that are necessary to produce these changes.

Previous reports have demonstrated that extremely low frequency (ELF) electromagnetic (EM) fields can alter the association of calcium ions with chick forebrain tissue in vitro [Bawin and Adey, 1976; Blackman et al, 1982]. For the purposes of this article, ELF denotes the frequency range from 1 to 300 Hz. The initial report [Bawin and Adey, 1976] demonstrated that a narrow frequency range of sinusoidal electric fields around 16 Hz, and within an intensity range bracketed by 10 and 56 $V_{p\text{-}p}/m$ (volts peak-to-peak per meter), was effective in causing a change in the calcium-ion association with brain tissue. Subsequently, Blackman et al [1982] independently demonstrated a similar intensity response for 16-Hz sinusoidal electromagnetic fields, using an entirely different exposure chamber. However, their more detailed study revealed, for the first time, two effective intensity ranges for induced efflux, at 5 to 7.5 $V_{p\text{-}p}/m$ and at 35 to 50 $V_{p\text{-}p}/m$, and ineffective intensity ranges bracketing those values at 1 to 3.5, 10 to 30, and 60 to 70 $V_{p\text{-}p}/m$. This unusual intensity response of tissue to ELF signals, demonstrating two clearly separated, effective intensity ranges, was consistent with the two effective power density ranges observed when the tissue was exposed to radiofrequency (RF) fields, amplitude modulated at 16 Hz [Blackman et al, 1980b, 1981; Joines et al, 1980]. A quantitative comparison cannot be made between the results obtained with ELF and modulated RF fields because the efficiency of demodulation is unknown [Blackman et al, 1982].

Although both the Bawin and the Blackman groups showed that 1-Hz and 30- or 32-Hz fields did not elicit a change in the association of calcium ion with brain tissue at the intensity that was effective for a 16-Hz field, no attempt was made to scan higher-order multiples of the basic frequency. In this study, we identify a higher frequency that exhibits an intensity response between 30 and 60 $V_{p\text{-}p}/m$, which is essentially identical to the response induced by the 16-Hz field. Using this result, we established an intensity optimum, which was then used to study a series of frequencies between 1 and 120 Hz. In addition, we describe the effects of 50-Hz and 60-Hz fields as well as 50-Hz amplitude-modulated (AM) 50-MHz RF carrier waves.

MATERIALS AND METHODS

The materials and methods used in this study are essentially identical to those reported in detail elsewhere [Blackman et al, 1982]. Only the basic outline and changes will be described here.

Exposure System

A transmission line (TEM cell) (Instruments for Industry model CC105S) terminated in a 50-Ω load (Narda model 375 NM) and modified for temperature control, served as the chamber to treat the samples with electromagnetic fields between 1 and 120 Hz (ELF); a water bath, maintained at the same temperature, was used for the control samples. The transmission line was aligned with its long axis

vertical; the steady-state magnetic field at the sample location had an inclination of 85°N and an intensity of 0.38 Gauss. For ELF-field exposure, a function generator (Wavetek model 186) supplied the sinusoidal signal to the transmission line. The signal intensity was monitored with a voltmeter (Ballentine model 3020A), and the frequency was monitored with a counter (Digitec model 8510A). In the text, the values of electric-field intensity are cited in units of volts peak-to-peak per meter (to convert these values to the more common term, volts root-mean-square per meter, divide by 2.828). Although the intensity values are cited for the electric-field component because it was easy to measure, in the radiation field propagating down the waveguide exposure system there is an accompanying magnetic-field component, whose magnitude, in amperes per meter, is equal to the electric-field intensity (Vrms/m) divided by 377, according to theory.

The exposure system for treatment of samples with a 50-MHz carrier wave, sinusoidally amplitude modulated at 50 Hz, has been previously described in detail [Blackman et al, 1979, 1980a]. A custom-made transmission line served as the exposure chamber, a signal generator (Wavetek model 3001) produced the RF carrier wave, and conventional instrumentation for power detection was used to measure the incident power. The characteristic response of the transmission line has also been described elsewhere [Weil, 1978]. The transmission line was aligned with its long axis vertical; the steady-state magnetic field at the sample location had an inclination of 60°N and an intensity of 0.38 Gauss, and was aligned perpendicular to the electric-field component of the RF signal.

Materials

Brain tissues were obtained from 1- to 7-day-old chicks (Gallus domesticus) purchased from North Carolina State University, Raleigh. The medium used in this study was composed of 155 mM NaCl, 5.6 mM KCl, 2.16 mM $CaCl_2$, 2.4 mM $NaHCO_3$, and 11.1 mM glucose. For the radioactive labeling of tissues, this medium was supplemented with calcium-45 ($^{45}Ca^{2+}$) at 1.0 μCi/ml (New England Nuclear, NEZ-013). All solutions were prepared on the same day they were used. The pH of the solutions was 7.8–8.0, and the temperature was 37 °C.

Preparation of Tissues

Four chicks were decapitated at a time, and the forebrains were removed and separated along the midline for use as treatment-control pairs. The pia mater remained on the uncut portions of each brain half. The tissues were placed in a medium containing radioactive calcium and incubated for 30 min at 37 °C. Following two successive rinses in unlabeled medium, the brain halves from each pair were separated and placed into individual polystyrene tubes, 1 ml of unlabeled medium was added to each tube, and the tubes were sealed with latex stoppers.

Exposure of Tissues

For treatment, samples in sealed tubes were placed in racks arranged symmetrically on either side of the center conductor in the transmission line. The axis of propagation was incident on the bottom of the test tubes, which were positioned vertically in the transmission line. The temperature within the transmission line was maintained at 37 °C. Tubes containing the corresponding paired brain halves were placed in a water bath at 37 °C for control conditions.

Four chick brains were treated at a time, either to the specified field conditions or to zero intensity fields (sham exposure).

Four exposed and four sham-exposed brain pairs were treated within 1 h and make up one replication of the basic experiment. Seven to nine replications were performed for each frequency and field intensity condition. Data were collected for each condition before a new condition was examined. A ten-tube configuration was used in the transmission line, with four tubes containing the brain half with medium and six tubes containing only an equivalent amount of unlabeled medium as described previously [Blackman et al, 1980a].

The tissues were exposed for 20 min to the ELF electromagnetic fields at the combinations of frequency and intensity given in Tables 1 and 2. The data in Table 2 were collected in the following time sequence: 60, 75, 90, 105, 15, 30, 1, 45, 120 Hz. Exposures to 50-MHz carrier wave, sinusoidally amplitude-modulated at 50 Hz, were conducted at power densities shown in Table 3.

Assay for Calcium-Ion Efflux

The amount of radioactive calcium released by the tissue during the treatment period was estimated by withdrawing 0.2-ml aliquots, combining the aliquot with 5 ml of scintillation cocktail (National Diagnostics, Liquiscint), and counting the combined sample in a liquid scintillation counter, using sample-channel ratios to monitor counting efficiency.

ANALYSIS OF DATA

The relative quantity of radioactive calcium ions released by each tissue pair was calculated by taking the ratio of the counts per minute in the treated sample compared to the control sample (V_t/V_c).

There were several replicates for each combination of frequency and intensity; therefore, the first statistical model investigated the influence of replicates, exposure groups, and replicate by exposure-group interactions. This model was analyzed by a two-way analysis of variance, which tested for the significance of each of the three factors. None of the combinations of frequency and intensity had a significant replicate or interaction effect. The statistical model was therefore reduced to one that considered only the influence of an exposure-group effect. This model was analyzed by a one-way analysis of variance, which tested the significance of the exposure-group effect.

It should be noted that our statistical model does not allow for a direct comparison of patterns; each frequency-intensity combination is treated individually. An analysis for patterns would introduce a time factor that would make an unknown contribution to the results. The appropriate experimental design for pattern analysis over the range of frequencies and intensities investigated in this report would require an experimental effort beyond our resources. The approach we have taken is a reasonable simplification, yet it limits any discussion of underlying patterns to heuristic arguments only.

RESULTS

The response of brain tissue to sinusoidal electromagnetic fields between 42 and 60 Hz is given in Table 1. The response is given as the mean ratio of radioactive

TABLE 1. Relative Quantity of Calcium Ions Released by Brain Tissue Pairs as a Function of Exposure Intensity, 42- to 60-Hz Fields

Frequency (Hz)	Intensity			Ratio, V_t/V_c[a]			
	$V_{p\text{-}p}$/m	V_{rms}/m	Treatment	N	Mean	SE	P
42	30	10.6	Sham	32	1.018	0.034	
			Exposed	32	1.068	0.038	0.334
	40	14.1	Sham	32	1.038	0.049	
			Exposed	32	1.099	0.051	0.389
	50	17.7	Sham	32	1.026	0.040	
			Exposed	32	0.990	0.039	0.529
	60	21.2	Sham	32	1.011	0.037	
			Exposed	32	1.030	0.036	0.716
45	30	10.6	Sham	32	1.008	0.045	
			Exposed	32	1.022	0.036	0.814
	35	12.4	Sham	32	0.995	0.046	
			Exposed	32	1.144	0.048	0.030*
	40	14.1	Sham	28	0.961	0.037	
			Exposed	28	1.122	0.052	0.016*
	45	15.9	Sham	28	0.971	0.032	
			Exposed	28	1.138	0.048	0.005**
	50	17.7	Sham	32	0.995	0.039	
			Exposed	32	1.191	0.054	0.005**
	55	19.4	Sham	32	0.981	0.039	
			Exposed	32	1.071	0.034	0.088
48	40	14.1	Sham	32	1.045	0.050	
			Exposed	32	1.140	0.040	0.140
50	30	10.6	Sham	32	1.051	0.046	
			Exposed	32	1.033	0.042	0.775
	35	12.4	Sham	28	1.041	0.042	
			Exposed	28	1.108	0.033	0.213
	40	14.1	Sham	32	1.014	0.053	
			Exposed	32	1.140	0.047	0.079
	45	15.9	Sham	27	0.994	0.041	
			Exposed	28	1.148	0.027	0.027*
	50	17.7	Sham	32	0.969	0.039	
			Exposed	32	1.116	0.042	0.013*
	55	19.4	Sham	32	1.017	0.037	
			Exposed	32	1.107	0.054	0.176
	60	21.2	Sham	28	1.004	0.045	
			Exposed	32	1.078	0.037	0.206
	65	23.0	Sham	32	1.017	0.040	
			Exposed	32	1.103	0.038	0.123
60	25	8.8	Sham	32	1.029	0.046	
			Exposed	32	1.039	0.047	0.874
	30	10.6	Sham	32	0.972	0.037	
			Exposed	32	1.077	0.043	0.068
	35	12.4	Sham	32	0.936	0.037	
			Exposed	32	1.088	0.039	0.007**
	40	14.1	Sham	28	0.963	0.043	
			Exposed	32	1.112	0.037	0.011*
	45	15.9	Sham	28	0.987	0.037	
			Exposed	28	1.063	0.042	0.182

[a]Ratio of counts per minute in the treated sample compared to the control sample.
*$P < 0.05$; **$P < 0.01$.

TABLE 2. Relative Quantity of Calcium Ions Released by Brain Tissue Pairs as a Function of Exposure to ELF Fields at 42.5 V_{p-p}/m†

Frequency (Hz)	Treatment	Ratio, V_t/V_c[a] N	Mean	SE	P
1	Sham	32	0.995	0.041	
	Exposed	32	1.036	0.041	0.490
15	Sham	32	1.013	0.042	
	Exposed	36	1.173	0.034	0.004**
30	Sham	32	1.033	0.032	
	Exposed	32	1.107	0.035	0.120
45	Sham	32	0.990	0.032	
	Exposed	32	1.181	0.052	0.003**
60	Sham	32	0.998	0.040	
	Exposed	32	1.136	0.044	0.024*
75	Sham	32	0.992	0.033	
	Exposed	32	1.148	0.044	0.006**
90	Sham	28	0.984	0.035	
	Exposed	32	1.094	0.036	0.035*
105	Sham	32	0.981	0.029	
	Exposed	32	1.143	0.041	0.002**
120	Sham	32	1.037	0.035	
	Exposed	32	1.084	0.035	0.342

†Equivalent to 15 V_{rms}/m.
[a]Ratio of the counts per minute in the treated sample compared to the control sample.
*P < 0.05; **P < 0.01.

TABLE 3. Relative Quantity of Calcium Ions Released by Brain Tissue Pairs as a Function of Intensity of 50-MHz Carrier Waves, Sinusoidally Amplitude Modulated at 50 Hz

Power density mW/cm^2	Treatment	Ratio, V_t/V_c[a] N	Mean	SE	P
1.56	Sham	32	0.964	0.045	
	Exposed	32	0.965	0.045	0.989
1.86	Sham	32	0.928	0.040	
	Exposed	32	1.134	0.040	<0.001***
1.96	Sham	32	0.948	0.050	
	Exposed	32	1.166	0.053	0.004**
2.30	Sham	32	0.997	0.046	
	Exposed	32	1.066	0.049	0.306

[a]Ratio of the counts per minute in the treated sample compared to the control sample.
**P < 0.01; ***P < 0.001.

calcium ions released by the brain tissue pairs (V_t/V_c) with sample size (N) and standard error of the mean (SE), for the sham and exposed groups at each combination of frequency and intensity. The P values for the one-way analysis of variance are given in the last column of the table. A P value less than 0.05 was considered statistically significant. Exposure to 42-Hz fields produced no statistically significant alterations in the efflux of calcium ions from the tissues at any of the intensities tested. Exposure to 45-Hz fields produced statistically significant changes at 35, 40, 45, and 50 $V_{p\text{-}p}/m$; exposure to 50-Hz fields produced statistically significant changes at 45 and 50 $V_{p\text{-}p}/m$, and exposure to 60-Hz fields produced statistically significant changes at 35 and 40 $V_{p\text{-}p}/m$.

The response of brain tissue to 42.5 $V_{p\text{-}p}/m$ (15 V_{rms}/m) ELF fields at frequencies between 1 and 120 Hz is shown in Table 2. The data are presented in the same form as for Table 1. Statistically significant responses ($P < 0.05$) occurred at 15 Hz and at 45–105 Hz, with no effects ($P \geq 0.12$) at 1, 30, and 120 Hz.

The results of exposure to several power densities of RF radiation, amplitude modulated at 50 Hz, are shown in Table 3. Again the relative quantity of radioactive calcium ions released by the brain tissue pairs is presented in the same form as for Table 1. Two power densities, 1.86 and 1.96 mW/cm^2, produced statistically significant results ($P < 0.01$), whereas lower and higher power densities did not.

DISCUSSION

This study is an extension of our previous work [Blackman et al, 1982] in which we described the enhanced efflux of calcium ions from chick brain tissue, in vitro, caused by exposure to 16-Hz, sinusoidal electromagnetic fields. In the earlier study, two intensity ranges, 5–7.5 and 35–50 $V_{p\text{-}p}/m$, were effective in causing enhanced efflux. In this study, we used the middle of the wider 35–50 $V_{p\text{-}p}/m$ range to examine the response induced by various frequencies. The intensity values cited here are for the electric-field component in air in the absence of the samples. The values of the electric-field component in the medium and in the brain tissue, E_i, can be estimated by assuming a spherical sample and using the relationship $E_i = (3\ \omega\epsilon_o/\sigma_i)E_o$, where E_o is the electric-field intensity in air, ω is the angular frequency, ϵ_o is 8.85×10^{-12} F/m, and σ_i is the conductivity of either the medium (~ 1.5 S/m) or the brain tissue (~ 0.15 S/m). For example, for a 45-Hz signal, the reduction factor ($3\ \omega\epsilon_o/\sigma_i$) is 0.5×10^{-8} for the medium and 5×10^{-8} for the brain tissue. These electric-field values in the sample are well below those that could cause generalized heating of the tissue.

Our initial experimental approach was to characterize the intensity response at 42, 45, 48, 50, and 60 Hz. The 50- and 60-Hz frequencies were selected because of their obvious importance in the transmission of electric power throughout much of the world. The data in Table 1 show no field-induced efflux enhancement for a 42-Hz signal at intensities between 30 and 60 $V_{p\text{-}p}/m$. Thus, we have found no evidence for a single-intensity band that can induce enhanced efflux in the brain tissue samples at all frequencies between 16 and 45 Hz. However, the 45-Hz fields show an intensity response essentially identical to that produced by 16-Hz fields between 35 and 50 $V_{p\text{-}p}/m$. Because exposure at 48 Hz and 40 $V_{p\text{-}p}/m$ did not cause a significant enhancement in efflux ($P = 0.140$), it appears that 45-Hz fields are closer to the relative frequency maximum in this intensity region. The 50-Hz intensity response displays a shift to slightly higher values for enhanced efflux, whereas the 60-Hz intensity

response is shifted to slightly lower values. As noted in the section above on analysis of data, any discussion or comparison of response patterns is heuristic.

Nevertheless, the intensity response in the 40 $V_{p\text{-}p}$/m for 16-Hz and 45-Hz fields served as the basis for selection of 42.5 $V_{p\text{-}p}$/m as the optimum intensity to be used in an extended frequency study. From the 45-Hz response observed here, and on the 1-Hz and 30-Hz results of our previous work [Blackman et al, 1982], we selected multiples of 15 Hz to test for efflux alterations. The results show highly significant enhancements induced by 15-, 45-, 75-, and 105-Hz fields ($P \leqslant 0.006$)—that is, odd multiples of 15 Hz. From this perspective, the 50-Hz result could just be part of the effective frequency range associated with the 45-Hz peak. Significant enhancements were also caused by 60-Hz ($P = 0.024$) and 90-Hz ($P = 0.035$) fields, whereas 30-Hz fields did not cause significant enhancement ($P = 0.120$). Although the mean differences in efflux between exposed and sham treatments for 15, 45, 75, and 105 Hz, which ranged from 0.15 to 0.19, were not statistically distinct from the differences for 30, 60, and 90 Hz, which ranged from 0.07 to 0.14, perhaps owing to the introduction of the time factor mentioned in the analysis-of-data section, it is possible that these two frequency series represent the interaction of the electromagnetic fields with two different response sites or via two different mechanisms. In the one case, strong resonancelike interactions with a highly structured site could be responsible for the relatively sharp, frequency-specific responses based on a 15-Hz fundamental frequency; however, the reason for the effectiveness of only the odd multiples is not understood. Perhaps certain common features of excitable tissue are involved, because similar frequency-specific results have recently been reported for intact frog heart, in vitro [Schwartz et al, 1983]. The broader and slightly weaker response, indicated by the possible trend at 30 Hz and the significant responses at 60 Hz and 90 Hz, may reflect the response of a more heterogeneous class of structures common to brain and other, more diverse tissue types. A similar, broad frequency response has been observed by Lyle et al [1983], who reported a field-induced change in the cytolytic activity of T lymphocytes exposed to RF fields, amplitude modulated at 60 Hz, with lesser changes at 16 and 40 Hz and at 80 and 100 Hz. Of course, the similarity in radiological response parameters may be circumstantial. Additional work is needed to test this proposal and to identify structures as potential sites of interaction. Figure 1 summarizes the combinations of frequency and intensity of ELF signals that have been tested for efflux enhancement and the outcome of those tests.

Motivated by the significant tissue response to 50-Hz fields, we examined the influence of 50-MHz, RF carrier waves, amplitude modulated at 50 Hz, on the efflux of calcium ions from brain tissue. The selection of 50 MHz as the RF carrier frequency was based on previous experience at that frequency [Blackman et al, 1980b]. Our results show that 50-Hz AM causes enhanced efflux at slightly higher power densities than 16-Hz AM, where enhancement occurred at 1.44 and 1.67 MW/cm^2 [Blackman et al, 1980b]. This contrast is consistent with the shift in effective intensities observed for ELF fields in this study. The reason for the intensity shift is unknown. The sensitivity of brain tissue to 50-Hz fields, both when treated directly and when modulating RF carrier waves, may be a clue to explain the conflict between reports by Soviet scientists of RF-induced biological changes [McRee, 1980] and the inability of scientists in the United States to duplicate these findings. It is possible that power-line ripple is present on the RF signal (50 Hz) used by Soviet scientists as well as on some signals (60 Hz) generated in the United States. For example, Heynick et

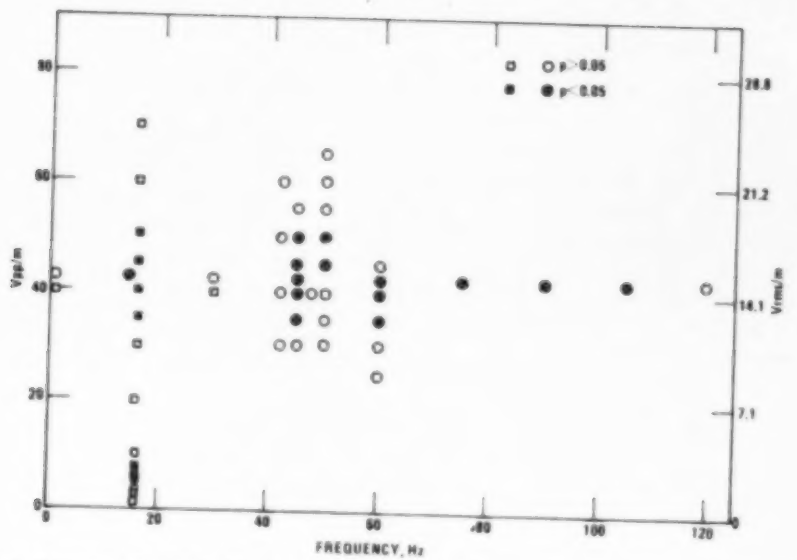

Fig. 1. Summary of results of ELF frequency and intensity combinations that have been tested for ability to cause calcium-ion efflux enhancement from brain tissue in vitro. Data from this study are denoted by circles; those from Blackman et al [1982] are denoted by squares.

al [1977] described a 60-Hz power-line-associated modulation on the signal from their magnetron source, which was obtained from a microwave oven [see also Anderstam et al, 1983]. It has been suggested that the spectral content of radiofrequency sources be included as part of standard characterization procedures [Ali and Weil, 1983]. Recently, the signal from one Soviet exposure system was found to have 50-Hz power-line-associated modulation (Bassen, personal communication). Although the 50-Hz modulation used in this study was greater than 90%, lower percentages may also be effective if the power density of the carrier waves is adjusted accordingly (Dutta, personal communication). The potential influence of ELF power-line ripple as a causative agent in RF studies deserves further investigation.

We have reported on many aspects of the phenomenon of field-induced calcium-ion efflux from brain tissues in vitro [Blackman et al, 1979, 1980a,b, 1981, 1982; Joines et al, 1981; Joines and Blackman, 1980, 1981]. During the course of these studies, we improved our experimental design and techniques to minimize the influences of extraneous variables and improve the statistical significance of the findings [see discussion sections in Blackman et al, 1980a,b, 1982]. However, additional work must be done to identify and characterize other variables that influence this phenomenon. For example, radiation-induced efflux has been shown to occur outside the normal pH range of 7.3–7.4 for cerebral spinal fluid; Bawin et al [1978] reported effects using a medium at pH 6.8, whereas this study demonstrates effects at a medium pH of 7.8–8.0. Although the underlying mechanism of action has not been established, several hypotheses have been advanced to guide future research [Adey 1981a,b; Blackman et al, 1981]. Our most recent work [Blackman et al, 1982] focused on the ELF range that was needed as modulation of the RF signal for the induction of enhanced efflux [Bawin et al, 1978; Blackman et al, 1979]. In this study, we observed enhanced efflux of calcium ions induced by 16-Hz signals in contrast to the reduced

efflux reported by Bawin and Adey [1976]. The reason for this difference is still unknown [see discussion in Blackman et al, 1982].

The purpose of this phenomenological report has been to define those conditions of frequency and intensity that are effective in inducing changes in brain tissue in vitro. These results can serve as a basis for the development of experimental protocols to examine the consequences of such exposure for other biological endpoints. A recent report by Adey and co-workers [1982], which demonstrated field-induced changes in calcium efflux for the cerebral cortex of an anesthetized cat, indicates that the results obtained with tissue from an avian species in vitro may be applicable to results obtained with a mammalian species in vivo. Other studies ranging from subcellular preparations [Lin-Liu and Adey, 1982] and cell cultures [Dixey and Rein, 1982] to animal behavior experiments [Gavalas-Medici and Day-Magdaleno, 1976] also support the general nature of biological responses to externally imposed electric, magnetic, and electromagnetic fields. Future studies should include attempts to determine the underlying reaction site and mechanism causing these phenomena, and to establish the physiological ramifications for the whole animal of such field-induced changes.

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